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Yield, Content, and Composition of Peppermint and Spearmints as a Function of Harvesting Time and Drying

Valtcho D. Zheljazkov,*,† Charles L. Cantrell,§ Tess Astatkie,# and Alex Hristov⊥

†North Mississippi Research and Extension Center, Mississippi State University, 5421 Highway 145 South, Verona, Mississippi 38879, United States, §Natural Products Utilization Research Unit, Agricultural Research Service, U.S. Department of Agriculture, P.O. Box 8048, University, Mississippi 38677, United States, #Department of Engineering, Nova Scotia Agricultural College, P.O. Box 550, Truro, Nova Scotia B2N 5E3, Canada, and ⊥Department of Dairy and Animal Science, Pennsylvania State University, University Park, Pennsylvania 16802, United States

Peppermint (*Mentha* × *piperita* L.) and spearmints ('Scotch' spearmint, *M.* × *gracilis* Sole, and 'Native' spearmint, *Mentha spicata* L.) are widely grown essential oil crops in more northern latitudes; however, there is limited information on how harvest time and drying influence peppermint and spearmint yield, oil composition, and bioactivity, when grown south of the 41st parallel. In this 2-year study, the effects of harvest time and drying on the yield, oil composition, and bioactivity of peppermint ('Black Mitcham' and 'B90-9'), 'Scotch' spearmint, and 'Native' spearmint were evaluated. Peppermint oil from the dried material had higher menthol and eucalyptol concentrations. Menthone in both peppermint cultivars decreased from harvest 1 (late June) to harvest 5 (late August) or 6 (early September), whereas menthol increased. (−)-Carvone in spearmints accumulated early, before flowering, allowing for early harvest. Oil yields from the dried spearmint biomass reached the maximum at harvest 3 (mid-July). The essential oil compositions of the four mint genotypes were similar to that of 11 commercially available oils, suggesting that these genotypes can be grown in the hot, humid environment of the southeastern United States. The antioxidant activities ($ORAC_{oil}$ values) of the essential oils were 4372, 1713, 1107, and 471 μmol of TE L^{-1} for 'Scotch' spearmint, 'Native' spearmint, peppermint, and Japanese cornmint (*Mentha canadensis*), respectively. The oils of the four mint genotypes did not affect ruminal fermentation in vivo, and did not exhibit antimicrobial, antileishmanial, or antimalarial activity at levels that would warrant bioassay-directed fractionation in a drug-discovery screening program. Specifically, the oils did not show greater than 50% growth inhibition against *Leishmania donovani*, *Plasmodium falciparum* clones D6 and W2, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Cryptococcus neoformans*, *Mycobacterium intracellulare*, or *Aspergillus fumigates* at 50 μg mL^{-1}.

KEYWORDS: *Mentha* × *piperita*; *Mentha* × *gracilis*; *Mentha spicata*; essential oil; Scotch spearmint; native spearmint; ginger mint; antioxidant; antileishmanial; antifungal; antimicrobial; ruminal fermentation

INTRODUCTION

Peppermint, 'Scotch' spearmint, and 'Native' spearmint are grown for the production of essential oil and for fresh or dried herbage throughout the world and are among the most important essential oil crops (*1*, *2*). Fresh and dried herbages are used for teas and to flavor foods, dishes, and beverages. Spearmint and peppermint essential oils are used extensively as aromatic agents in various products such as chewing gum, toothpaste, and mouthwashes, as well as in pharmaceuticals, confectionary, aromatherapy, as antimicrobial agents, and in ecofriendly pesticides (*2*, *3*). Peppermint and spearmint herbage, extracts, and essential oils have a long history of medicinal use for controlling a number of human diseases or alleviating ailments (*4*). Spearmint and peppermint essential oils (or individual oil constituents such as (−)-carvone, menthol, or others) exhibit antimicrobial properties (*5*−*11*).

Peppermint and spearmints are temperate plants, and peppermint especially requires long days to reach flowering and accumulate essential oil with desirable composition (*2*, *12*, *13*). Hence, both peppermint and spearmints are generally grown north of the 41st parallel in the northern hemisphere, although these species also are grown in Australia in the southern hemisphere (*2*). Due to increased market opportunities, the essential oil industry is looking for new peppermint and spearmint production areas in more southern latitudes in North America, where abundant irrigation and longer growing seasons (i.e., a greater number of frost-free days) may bring more harvests per season and thus

*Author to whom correspondence should be addressed [phone (662) 566-2201; fax (662) 566-2257; e-mail vj40@pss.msstate.edu].

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improve the overall economics for essential oil production (*3*). Recent research has demonstrated that peppermint and spearmint can be grown successfully far south of the 41st parallel, at latitudes of around 34° N in the southeastern United States (*14*, *15*).

Spearmints and peppermint are traditionally dried prior to steam distillation to decrease production costs and increase efficiency (*2*). There is no information on how the timing of harvest and drying would alter productivity, essential oil content, composition, and yields of the major oil constituents of spearmints and peppermint grown in the hot, humid environment of more southern latitudes. Furthermore, it is important to compare peppermint and spearmint oils produced south of the 41st parallel to commercially available oils. The objectives of this study were (1) to evaluate the effect of harvest time and drying on the productivity, oil content, composition, and yield constituents of peppermint 'Black Mitcham' and 'B90-9' and of 'Scotch' and 'Native' spearmints in the hot, humid climate of Mississippi; (2) to test the bioactivity of the oils from this study; and (3) to compare their composition to those of commercially available oils.

MATERIALS AND METHODS

Plant Materials and Growing Conditions. Commercial peppermint and spearmint varieties are propagated exclusively vegetatively, due to their hybrid nature (*2*, *16*, *17*). To ensure no pressure from diseases and pests, certified and virus-free planting material (propagated through tissue culture) of cultivars 'Black Mitcham' and 'B90-9' of *Mentha* × *piperita* L., 'Scotch' of *Mentha* × *gracilis* Sole (syn. *Mentha cardiaca* L.), and 'Native' of *Mentha spicata* L. (syn. *Mentha viridis* L.) were purchased from Summit Plant Laboratories, Inc. (Fort Collins, CO). 'Black Mitcham' is the main peppermint and 'Scotch' and 'Native' are the main spearmint varieties used for commercial production of essential oil in North America, Australia, and other places.

A field experiment was conducted in 2007 and 2008 at the North Mississippi Research and Extension Center in Verona, MS (34.16° N, 88.73° W, 80 m asl). The soil at the experimental site was Quitman sandy loam (fine-loamy, siliceous, semiactive, thermic, Aquic Paleudult) with 1.15% organic matter, 6% clay, 55% silt, and 38% sand, a pH of 6.4, and concentrations of available nutrients as follows: P, 63 kg ha^{-1}; K, 59 kg ha^{-1}; Ca, 1912 kg ha^{-1}; Mg, 81 kg ha^{-1}; Zn, 1.4 kg ha^{-1}; S, 130 kg ha^{-1}; and Na, 118 kg ha^{-1}.

Land preparation and weed control of the experimental site and the drip tape irrigation systems were as described previously (*14*, *15*). Peppermint and spearmint plants were transplanted on May 4, 2007, and kept as perennial crops for the next cropping season. The planting density was 30 cm within rows and 30 cm between rows. After the first cut, the plant density increased greatly due to the sprouting of buds on the underground rhizomes. For essential oil extraction, approximately 1 m^2 was harvested from each experimental plot. The necessary nutrients were provided through the application of slow-release fertilizer (Osmocote Plus15N-9P-12K; Scotts-Sierra Horticultural Products Co., Marysville, OH), calculated to provide 120, 72, and 96 kg ha^{-1} of N, P, and K for each of the growing seasons.

Harvest dates of peppermint plants in 2007 were June 26 (harvest 1), July 12 (harvest 2), July 24 (harvest 3), August 6 (harvest 4), August 23 (harvest 5), and September 4 (harvest 6), and those of spearmint plants were June 26 (harvest 1), July 12 (harvest 2), July 24 (harvest 3), August 6 (harvest 4), August 23 (harvest 5), and September 4 (harvest 6). These harvest dates correspond to 53, 69, 81, 94, 111, and 123 days after transplantation. In 2008, peppermint and spearmint plants developed earlier and were harvested 7−15 days earlier for the respective harvesting times.

Plants were harvested using a hedge trimmer by cutting the plants approximately 10 cm above the soil surface. Fresh biomass weight was measured; half of the harvested biomass was separated and dried at 40 °C for 2 weeks, and both fresh (500 g) and dried (250 g) subsamples were steam-distilled for the essential oil extraction. The steam distillation was performed for 60 min in a 2 L steam distillation Clevenger type apparatus (*14*, *15*). The essential oil was measured and calculated as grams of oil per gram of dried plant tissue. Samples from every treatment and replicate were collected and analyzed separately for oil composition.

GC-MS Analysis and Conditions. Chemical standards, peppermint, and spearmint oils were analyzed by gas chromatography−mass spectrophotometer (GC-MS) analyses on a Varian (Palo Alto, CA) CP-3800 GC coupled to a Varian Saturn 2000 MS/MS. The GC-MS methods for analysis and conditions are nearly identical to those previously described by Zheljazkov et al. (*21*). Briefly, the GC had a Varian CP-Sil 8CB fused silica capillary column (30 m × 0.25 mm, 0.25 μm) under the following temperature program: injector temperature, 240 °C; column temperature, raised from 60 to 240 °C at 3 °C/min and then held at 240 °C for 5 min; carrier gas, He; injection volume, 1 μL (splitless). The MS mass had a prescan ionization time of 100 μs, an ion trap temperature of 150 °C, a manifold temperature of 60 °C, and a transfer line temperature of 170 °C.

Quantitative Analysis of Essential Oils. The GC-MS analysis of the essential oils from all treatments and replicates was conducted essentially as described earlier (*14*, *15*). Eucalyptol, (−)-menthol, (−)-menthone, and (+)-menthofuran are the major constituents of peppermint oil, and for this reason and others they were chosen to be quantified. Similarly, (−)-carvone, eucalyptol, and (*R*)-(+)-limonene were chosen to be quantified in spearmint oil. Commercial standards eucalyptol, (−)-menthol, (−)-menthone, (+)-menthofuran, (−)-carvone, and (*R*)-(+)-limonene were obtained from Fluka (Buchs, Switzerland). With five concentration points, an external standard least-squares regression was performed. All analytes were used to generate separate calibration curves. Linearity was imposed by independently using response factors (RFs) and regression coefficients. The response factors were calculated using the equation RF = DR/*C*, where DR is the detector response in peak area (PA) and *C* is the concentration of the analyzed standard or oil. The chromatograms for each of the oils from the field experiments and the commercial mint oil samples were compared with the chromatograms from standard injections. The target peaks were confirmed by both retention time and mass spectra. Confirmed integrated peaks were then used to determine the percentage of each chemical constituent in the essential oil. The RF of the target chemical constituent was used to determine the percentage of that constituent in each essential oil sample using the equation (PA/RF/*C*) × 100 = % (peak area/response factor/concentration).

Antimicrobial, Antimalarial, and Antileishmanial Activity, Cytotoxicity, and Antioxidant Activity of Essential Oils from Mint Genotypes. Representative essential oil samples from the two peppermint genotypes and two spearmint genotypes, as well as from two genotypes of Japanese cornmint (*Mentha canadensis* L.; grown in the same field and extracted the same way) were tested for antimicrobial (against *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Cryptococcus neoformans*, *Mycobacterium intracellulare*, or *Aspergillus fumigates*) and antimalarial (*Plasmodium falciparum* clones D6 and W2) activity as described previously (*18*). The same oils from the four mint species were screened for in vitro cytotoxicity against mammalian kidney

Table 1. Mean Biomass Yield from Dry and Fresh Materials at Six Harvest Times for the Two Spearmint Genotypes and Mean (−)-Carvone Concentrations from Four Combinations of Cultivar and Material at Six Harvest Times of the Two Spearmint Genotypes

biomass yields (kg/ha)			(−)-carvone concentration (%)			
	material		dry		fresh	
harvest[a]	dry	fresh	Native	Scotch	Native	Scotch
1	2192 g[b]	12722 c	60.9 a	52.1 abcd	44.3 cdef	51.4 abcd
2	3685 f	16654 b	55.9 abc	53.9 abc	48.7 bcde	48.7 bcde
3	4588 ef	16923 b	61.8 a	45.2 cdef	41.2 def	59.9 ab
4	5470 de	19673 b	54.0 abc	44.4 cdef	35.4 f	52.5 abcd
5	5379 de	20388 b	60.7 a	47.5 bcde	39.2 ef	47.8 bcde
6	6376 d	26460 a	44.9 cdef	41.5 def	62.6 a	52.6 abcd

[a] Harvest dates of spearmint plants were June 26 (harvest 1), July 12 (harvest 2), July 24 (harvest 3), August 6 (harvest 4), August 23 (harvest 5), and September 4 (harvest 6). [b] Means followed by the same letter are not significantly different.

fibroblasts (VERO) and kidney epithelial cells (LLC-PK11) by the neutral red assay method (*18*, *19*) and for antileishmanial activity in vitro on a culture of *Leishmania donovani* promastigotes using the assay of Mikus and Steverding (*20*). The screening was performed in an in-house testing facility at the National Center for Natural Products Research at the University of Mississippi. Furthermore, the antioxidant activities of representative essential oil samples from the peppermint and spearmint species from this study as well as oils from Japanese cornmint were tested for antioxidant activity using the $ORAC_{oil}$ assay method described previously (*22*). The $ORAC_{oil}$ method is suitable for essential oils and other lipids that are difficult to dissolve in aqueous solutions.

Screening of Mint Oils for Ruminant Digestibility. Essential oils from peppermint and spearmint genotypes from this study along with oils from two genotypes of *M. canadensis* L. grown in the same field and extracted the same way were examined as part of a larger experiment screening 88 essential oils for their effects on methane production, ruminal neutral detergent fiber (NDF) digestibility, and ruminal fermentation in vitro as described previously (*23*). Essential oils were tested at three application levels: 10, 50, and 100 mg L^{-1} final medium concentrations. The effects of essential oils on fermentation and methane production were evaluated in 6 h incubations, and the effect on NDF digestibility was assayed in 24 h incubations.

Statistical Analyses. Statistical analyses were conducted on the biomass yield, oil content, and oil yield for all four cultivars (genotypes), (−)-carvone, eucalyptol, and limonene concentrations for the two spearmint cultivars, and menthol, menthone, eucalyptol, and menthofuran concentrations for the two peppermint cultivars that were collected at six harvests in six combinations of block (B1, B2, B3) and year (2007 and 2008). Biomass and oil yields and some oil compositions were analyzed as repeated measures in a two-factor (cultivar and material) factorial with six blocks, whereas the other oil compositions measured from fresh material were analyzed as repeated measures in a randomized complete blocks design. The analysis of variance (ANOVA) was performed using the Mixed Procedure of SAS (*24*), and further multiple means comparison was performed for significant (P value < 0.05) and marginally significant (P value between 0.05 and 0.1) effects by comparing the least-squares means of the corresponding treatment combinations using the LSmeans statement of Proc Mixed with the PDIFF option to produce P values for all pairwise differences. Letter groupings were generated using the 5% level of significance. For each response, the validity of model assumptions on the error terms was verified by examining the residuals as described previously (*25*).

Significant interaction with harvest effects were further investigated to see if the relationship between harvest and the corresponding response variables could be described using either a nonlinear regression model or a third-order polynomial regression model. Some of the relationships were described adequately by a three-parameter Gompertz regression model (eq 1) or a four-parameter logistic regression model (eq 2) for *M.* × *piperita*. Some of the relationships for all four genotypes were described adequately by a third-order polynomial regression (eq 3):

$$Y = \theta_1 e^{-e^{-\left(\frac{X-\theta_2}{\theta_3}\right)}} + \varepsilon \quad (1)$$

$$Y = \theta_0 + \frac{\theta_1}{1 + \left(\frac{X}{\theta_2}\right)^{-\theta_3}} + \varepsilon \quad (2)$$

$$Y = \beta_0 + \beta_1 X + \beta_2 X^2 + \beta_3 X^3 + \varepsilon \quad (3)$$

The parameter θ_1 in both the Gompertz (eq 1) and logistic (eq 2) models represents the upper asymptote value achieved at the largest harvest value. For both models, θ_2 and θ_3 adjust the shape of the curve by controlling the inflection point and the slope. The sign of θ_3 for the logistic model indicates whether it is a decay (negative) or growth (positive) function, and its magnitude indicates the rate of decay or growth. θ_0 for the four-parameter logistic model represents the lower asymptote of the growth curve. The parameters for the third-order polynomial regression (eq 3) are not physically meaningful. They simply adjust the shape of the curve.

The nonlinear regression analysis was completed using the NLIN Procedure of SAS, and the figures as well as the third-order polynomial fits were performed using Minitab 15 software (Minitab, State College, PA).

RESULTS AND DISCUSSION

Statistical analyses showed that there were differential responses of yields, oil content, and oil composition of both spearmint and peppermint cultivars with significant main and interaction effects (ANOVA P values not shown). Multiple means comparison results discussed below are based on the comparison of treatment combinations stemming from the highest order significant interaction effects. However, when a factor that is not involved in a significant interaction effect is significant, its levels were compared.

Spearmints. Overall, 'Scotch' spearmint had a higher concentration and greater yield of limonene than 'Native' spearmint (Supporting Information Table 1). Oil content in the dried biomass material was twice that in the fresh biomass material (Supporting Information Table 1) due to water evaporation during drying, which is why producers prefer to dry mint biomass prior to extraction (*2*, *4*). The biomass yields of the two spearmint genotypes, either dried or fresh, were not significantly different between the genotypes (Supporting Information Table 2). Overall, the fresh biomass yields of the two spearmint genotypes increased with a delay in harvesting time (**Table 1**). The concentration of (−)-carvone in 'Native' spearmint oil extracted from dried biomass decreased at harvest 6 (**Table 1**). (−)-Carvone concentration in 'Scotch' spearmint oil extracted from dried biomass showed a similar pattern, but the highest value was achieved at harvest 2. However, (−)-carvone concentration varied differently in oils extracted from fresh biomass. Overall, the concentration of (−)-carvone varied from 35 to 62% in 'Native' spearmint oil and from 42 to 60% in 'Scotch' spearmint oil (**Table 1**).

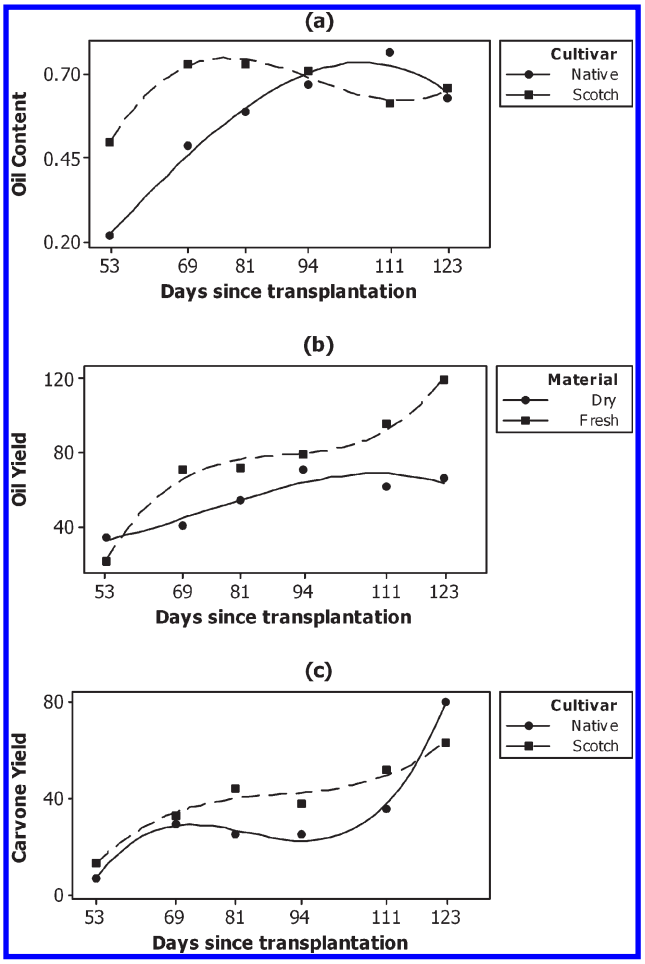

Figure 1. Fitted third-order polynomial regression of (**a**) oil content (%), (**b**) oil yield (kg ha^{-1}), and (**c**) carvone yield (kg ha^{-1}) on the number of days since transplantation for the two spearmint cultivars (genotypes) and the two materials.

Oil content of 'Native' spearmint increased gradually until harvest 3, whereas the oil content of 'Scotch' spearmint did not change significantly with harvesting time (**Figure 1a**). Generally, oil yields from the fresh biomass of the two spearmint genotypes increased until harvest 6, whereas oil yields from the dried biomass reached their maximum at harvest 3 (**Figure 1b**). Because growers prefer to extract spearmint oil from the dried biomass, harvest of spearmint in the southeastern United States should be done earlier, at harvest 3 (mid-July). The concentration of eucalyptol varied unequally in the two spearmint genotypes as a function of drying (Supporting Information Figure 1). (−)-Carvone yield was low at harvest 1, increased and reached a plateau, and then reached the highest values at harvest 6 (**Figure 1c**). The highest (−)-carvone yield was achieved at harvest 6 of 'Native' spearmint (**Figure 1c**).

Five spearmint essential oils produced in the United States and in China and commercially available on the U.S. market were purchased and analyzed (using the same methods and conditions as for field-grown material) (Supporting Information Table 3). In most instances, the spearmint oils from this experiment in Mississippi had greater concentrations of limonene and (−)-carvone than the commercially available oils. Hence, spearmint oils produced under the hot, humid conditions of the southeastern United States should be marketable.

The essential oil profile of spearmints from this study was similar to previous reports from the United States (*2*, *14*, *26*) and other countries (*1*, *6*, *11*, *27*−*30*). Under the environmental conditions of the southeastern United States, (−)-carvone accumulates in significant concentrations early in spearmint development, allowing for early harvest and presumably an additional one or two harvests per cropping season. When 'Native' spearmint is harvested early, drying of plant material increases (−)-carvone concentration in the oil. If it is harvested late (at the end of flowering), then it should be distilled fresh to obtain the highest (−)-carvone concentration in the oil. The highest (−)-carvone concentration in 'Scotch' spearmint oil would be obtained when plants are harvested at the beginning of flowering (mid-July) and the material is distilled fresh.

Table 2. Mean Biomass, Oil, Menthol, and Eucalyptol Yields for the Two Peppermint Cultivars

cultivar	biomass yield (kg/ha)	oil yield (kg/ha)	menthol yield (kg/ha)	eucalyptol yield (kg/ha)
B90-9	10166 a[a]	67.3 a	19.8 a	2.68 a
Black Mitcham	9244 b	55.2 b	16.1 b	2.14 b

[a] Means followed by the same letter are not significantly different.

Peppermints. 'B90-9' provided greater biomass and oil, menthol, and eucalypthol yields than 'Black Mitcham' (**Table 2**). As expected, the overall oil content in the dry biomass (0.985%) was almost 4-fold higher than that in fresh biomass (0.262%), confirming the practical significance of drying biomass prior to oil extraction (*2*, *4*). Drying also increased the eucalyptol concentration in the oil. Menthol concentration was higher in oil from the dry biomass of the two cultivars and lowest in oil from 'Black Mitcham' fresh biomass (Supporting Information Table 4). Both fresh and dried biomass yields were lowest during harvest 1 and were highest during harvest 6, whereas oil content reached a peak at harvest 4 and subsequently declined slightly by harvest 6 (**Table 3**).

The change in oil, menthol, and eucalyptol yields as a function of harvest was adequately modeled by a three-parameter Gompertz model (**Figure 2**). Parameter estimates of the model indicated the maximum achievable yield (per harvest but not per year) for oil, menthol, and eucalyptol were approximately 83, 25, and 3 kg ha^{-1}, respectively (data not shown).

The relationships between menthone concentration and menthofuran yield for 'B90-9' and menthofuran concentration for the two peppermint cultivars and the two materials were almost perfectly modeled by a four-parameter logistic regression model (**Figure 3**). The model for menthone concentration represents decay, whereas the model for the other responses represents growth (**Figure 3**). The parameter estimates indicate that the maximum achievable menthone and menthofuran concentrations in oils of 'B90-9' and 'Black Mitcham' and menthofuran concentration in oil extracted from dried and fresh biomass would be approximately 37%, 14.6%, 12.2%, 14.8%, and 12.9 kg ha^{-1}, respectively. The maximum yield of menthofuran from 'B90-9' was estimated to be approximately 16 kg ha^{-1}. Overall, the menthone concentration in oil of both peppermint cultivars decreased steadily from harvest 1 to harvest 5 or 6 (**Figure 3**). In contrast, the menthofuran concentration of the oil from dried biomass or from 'Black Mitcham' grew at the fastest rate, whereas the growth rate for the other three responses was moderate (**Figure 3**). Although menthone concentration in oil decreased at almost every subsequent harvest (**Figure 3**), menthone yield was higher at harvest 2 and then declined again in 'Black Mitcham' but increased in 'B90-9' at harvest 6 (Supporting Information Figure 2).

Comparison of peppermint oils from this study with six commercially available peppermint oils demonstrated that the oils produced in Mississippi had a composition similar to that of the Prime Idaho type oil and better than the composition of organic oil from Hungary (Supporting Information Table 5).

Table 3. Mean Biomass Yield (from Dry and Fresh Materials), Oil Content, Menthol Concentration, Eucalyptol Concentration, and Menthofuran Yield ('Black Mitcham' Cultivar) for the Six Harvest Times of the Two Peppermint Cultivars

	biomass yield (kg/ha)					
harvest[a]	dry	fresh	oil content (%)	menthol concentration (%)	eucalyptol concentration (%)	menthofuran yield, BM (kg/ha)
1	1903 g[b]	10754 c	0.36 c	31.0 ab	3.75 b	1.05 e
2	3999 f	17588 b	0.56 b	28.8 bc	4.83 a	4.44 de
3	5508 e	21087 b	0.55 b	32.8 a	4.58 a	6.17 cd
4	5675 e	22743 b	0.62 a	33.5 a	4.63 a	8.60 bc
5	5772 e	22444 b	0.58 ab	27.9 c	3.98 b	10.91 ab
6	7585 d	32481 a	0.54 b	31.3 ab	4.01 b	14.85 a

[a] Harvest dates of peppermint plants in 2007 were June 26 (harvest 1), July 12 (harvest 2), July 24 (harvest 3), August 6 (harvest 4), August 23 (harvest 5), and September 4 (harvest 6). [b] Within each response, means followed by the same letter are not significantly different at the 5% level.

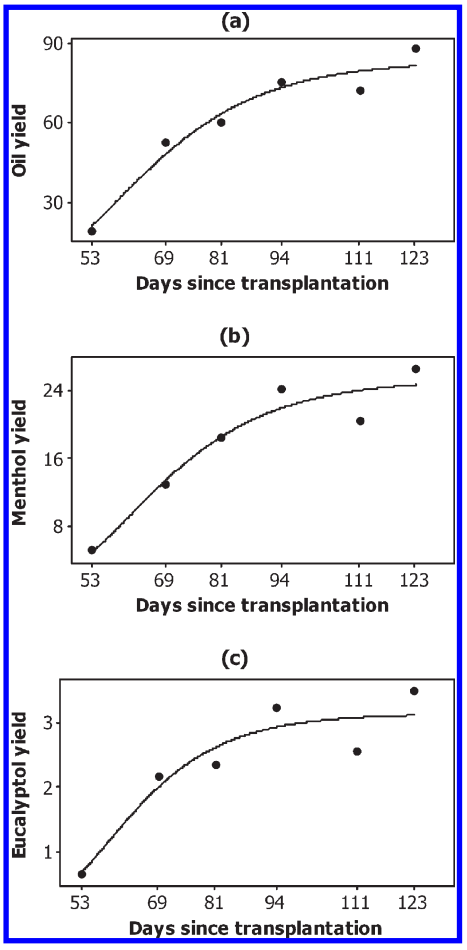

Figure 2. Fitted three-parameter Gompertz regression model of (**a**) oil, (**b**) menthol, and (**c**) eucalyptol yield (kg ha^{-1}) on the number of days since transplantation for the peppermint cultivars.

The peppermint essential oil profile from this study was similar to that given in previous papers (*4*, *7*, *15*, *31*, *32*), and the results agree with the current understanding of peppermint oil synthesis, composition, and accumulation (*4*, *33*−*36*). Menthol synthesis is an eight-step biochemical reaction starting with geranyl diphosphate in the plastids (*33*−*35*). The intermediate product (+)-pulegone can be converted into (+)-menthofuran (*34*), but menthofuran is a final product and cannot be converted to menthol. Both pulegone and (+)-menthofuran tend to accumulate under adverse environmental conditions (*34*) and are considered as hepatotoxic compounds by the Committee of Experts on Flavoring Substances (CEFS) of the Council of Europe (*37*). The CEFS proposed limits for the concentration of pulegone and (+)-menthofuran in various foods and beverages (*37*). Hence, peppermint oils with approximately 40% menthol and lower concentrations of pulegone and (+)-menthofuran are of higher quality and may bring higher prices to primary producers. Previous research indicated that (−)-menthone concentration reaches a peak in the early stage of peppermint development, perhaps around 12 days after leaf initiation (*33*). After that, there is increased production of menthone reductase, which mediates the conversion of (−)-menthone into (−)-menthol. However, results from this study suggest that the menthol biosynthesis may also be affected by environmental conditions. Most of the previous research on peppermint has been conducted north of the 41st parallel and even up to 63.6° N and 18−20 h day length (*36*), which is not typical for peppermint production areas and results in an atypical peppermint oil profile. Current understanding is that peppermint could not be grown south of the 41st parallel because it may not reach flowering (*12*, *13*, *38*). However, our 2-year field studies at three locations in Mississippi (in Verona, 34.16° N, 88.73° W; Stoneville, 33.42° N, 90.94° W; and Crystal Springs, 32° N, 90.35° W) demonstrated that peppermint will form flowers at latitudes around 32−34° N, indeed, with more prolific flowering at 34° N than at 32° N .

Effect of Essential Oils on Ruminant Digestibility. Methane production was not affected by any of the tested essential oils (Supporting Information Table 6). *Mentha arvensis* 'Arvensis 3′ and *M. spicata* significantly increased propionate concentration compared with the blank. Both essential oils also significantly increased butyrate concentration. The digestibility of NDF, however, was significantly reduced by *M. arvensis* 'Arvensis 3′ essential oils compared with the blank. *M. piperita* significantly increased ammonia concentration in the incubation medium. The trend for increased propionate (and butyrate) and decreased fiber digestibility with *M. arvensis* 'Arvensis 3′ may indicate a shift in microbial fermentative activities and possibly inhibition of ruminal protozoa (*39*). Overall, ruminal effects of the essential oils were subtle and unlikely to significantly affect ruminal fermentation in vivo.

Antioxidant Activity of the Essential Oils. The ORAC analyses indicated differential antioxidant activity among essential oils from the three spearmint and peppermint species used in this study and from Japanese cornmint. The $ORAC_{oil}$ values of the essential oils were as follows: 'Scotch' spearmint, 4372 μmol of TE L^{-1}; 'Native' spearmint, 1713 μmol of TE L^{-1}; peppermint oil, 1107 μmol of TE L^{-1}; and Japanese cornmint, 471 μmol of TE L^{-1}. In comparison with the antioxidant activities of some other oils measured using the $ORAC_{oil}$ method (*22*), the antioxidant activity of 'Scotch' spearmint was similar to that of cold-press organic soybean oil (4383 μmol of TE L^{-1}), the antioxidant activities of 'Native' spearmint and peppermint oils were lower than that of cold-press organic hazelnut oil (2521 μmol of

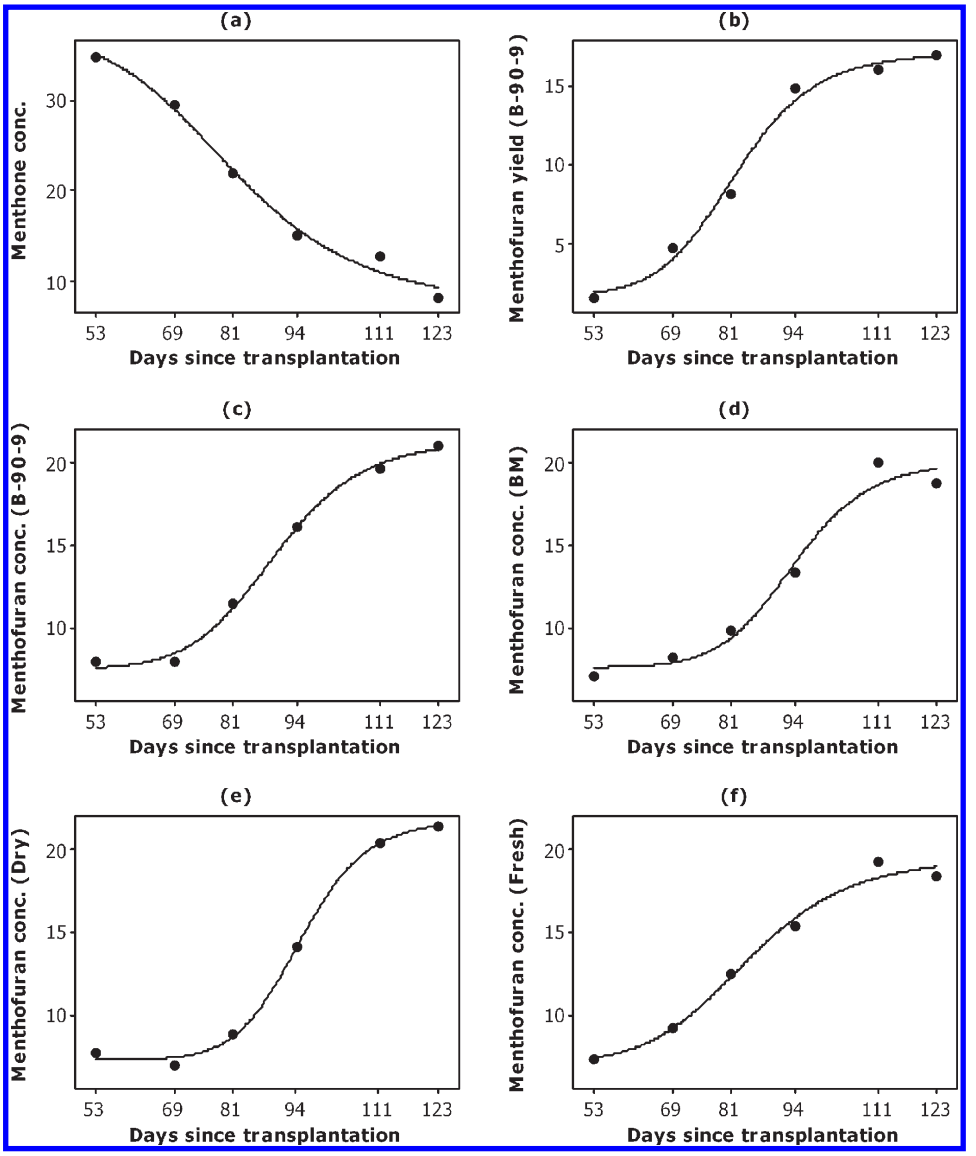

Figure 3. Fitted four-parameter logistic regression model of (**a**) menthone concentration (%), (**b**) menthofuran yield (kg ha^{-1}), and (**c**−**f**) menthofuran concentration (%) on the number of days since transplantation for the two materials and the two cultivars of peppermint.

TE L^{-1}) but higher than that of cold-press avocado oil (580 μmol of TE L^{-1}), and the antioxidant activity of Japanese cornmint oil was between those of avocado and cold-press organic stripped corn oil (332 μmol of TE L^{-1}). The essential oil of *M. canadensis* at concentrations of 365 μg mL^{-1} for DPPH and 0.3 μg mL^{-1} for OH exhibited >50% neutralization of respective oxidative radicals, and the antioxidant capacity of the oil was greater than that of quercetin (*40*). In another study of essential oil from *M. viridis* (*M. spicata*), the antioxidant activity measured by ABTS assay showed IC_{50} values of 195.1 ± 4.2 mg L^{-1} and the DPPH assays showed moderate IC_{50} (3476.3 ± 133 mg L^{-1}) (*8*). Although these different antioxidant analyses are difficult to compare, it is evident that spearmint essential oils have high antioxidant activity and peppermint oil has greater antioxidant activity than Japanese cornmint oil.

Antimicrobial, Antileishmanial, and Antimalarial Activity of the Spearmint Oils from This Study in Comparison with *M. canadensis* and *M.* × *piperita* Essential Oils. The essential oils of *M. spicata* and *M.* × *gracilis* from this study as well as representative essential oils of *M.* × *piperita* and *M. canadensis*, grown in the same field and extracted in the same way, did not show greater than 50% growth inhibition at 50 μg mL^{-1} against *L. donovani*, *P. falciparum* clones D6 and W2, *C. albicans*, *E. coli*, *P. aeruginosa*, *C. neoformans*, *M. intracellulare*, or *A. fumigates*. The inhibition of *P. falciparum* D6 clone was 9−10% by Japanese mint oil, 4−19% by peppermint oil, 23% by 'Native' spearmint oil, and 7% by 'Scotch' spearmint oil. Also, peppermint essential oils showed 6−18% inhibition of *C. albicans* and 26−29% inhibition of *C. neoformans*, and 'Native' spearmint showed 25% inhibition of *C. neoformans*. However, all activities were below the threshold required for further secondary assays as required by the National Center for National Products Research procedures.

Our results contradict earlier reports on the antibacterial or antifungal effect of spearmint and peppermint oils (*7*−*9*), most probably due to differences in the methods and concentrations used in the studies. Mkaddem et al. (*8*) reported that the essential oil of *M. viridis* (*M. spicata*) exhibited antimicrobial activity against *Listeria monocytogenes* and *Klebsiella pneumoniae* but not against *E. coli*, and they found significant antifungal activity of *M. viridis* essential oil. Iscan et al. (*7*) found that peppermint essential oils from different origins exhibited antimicrobial activity, which the authors attributed to menthol.

This is the first comprehensive study on how harvesting and postharvest management would affect the essential oil composition of commercial varieties of peppermint and spearmint, as well as the first to compare antioxidant, antimicrobial, antileishmanial, and antimalarial activities of oils from different mint species produced in the hot, humid conditions of the southeastern United States. Comparison of the peppermint and spearmint oil compositions from this study with those of 11 commercially available peppermint and spearmint oils demonstrated that peppermint and spearmint produced in the hot, humid climate of the southeastern United States would be marketable.

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Supporting Information Available: Additional tables and figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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